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NUTRITION REVIEWS

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NUTRITION REVIEWS

VOL. 5 SEPTEMBER, 1947 No. 9

WHAT PROGRESS IN NUTRITION?

Beginning early in the twentieth century the science of nutrition has undergone a renaissance and an expansion scarce paralleled by that of any other branch of human knowledge except physics. Some of the methods of biochemistry which were invented largely under the spur of need to know the vitamins, have also revealed many secrets of metabolism and enzymology and have helped to bring to use some of the most modern and effective drugs, notably the antibiotics. No one will dispute that there has been conspicuous progress in the science of nutrition. It still goes on rapidly.

What of the application of this science to human feeding?

In diagnosis and therapy of nutritional disease advances have been notable, though there is still urgent need for more knowledge and for expansion of this knowledge within the medical profession. However, there lies beyond the reach of the physician and of healing art the vast field of human feeding which is mainly in the hands of industry and of the housewife. It is to these we must look chiefly not only for prophylaxis against deficiency disease, but for the more positive good of vigorous nutritional health.

Industry is an extremely important agency for better feeding. We look to it for more efficient and cheaper methods of production. These yearly bring a new variety of foods within reach. We look to it by no means vainly to stretch the geographic and seasonal availability of foods by new methods of preservation and refrigeration. Even advertising makes an important contribution though perhaps not economically. An example is the expansion of the use of citrus and tomato juices as before-meal appetizers. Some of us can recall when the practice was extremely rare. On the whole, food industry can be said to be alert to the possibilities of popularizing its products through use of scientific knowledge.

So also the housewife within the lesser means at her disposal. Especially during the war years with all their emphasis upon selection, use, and rationing of foods, scientific knowledge has found a warm and often effective welcome within homes. Red Cross and other local women's groups, state and federal agencies, the promotional departments of food industries and of public utilities have all contributed to this. Above all, the expanding incomes for labor in war industries made possible increasing purchase of protective foods. Nationally, nutrition in America is substantially better than before the war.

However, it is characteristic of the methods of both industry and of popular education that they take effect most readily in spots of less acute need. It is the alert, the well-educated, the economically favored housewife who responds promptly to new and attractive food products and to information about foods and feeding. It is the less well-informed, the isolated and remote family which learns slowest and avails itself least of technologic advances. The benefits start largely in nonmarginal homes, thence filter down through the strata of society and often fail to reach large areas at the lower levels.

Government and charity seek to lessen this sinister selectivity by conduct of neighborhood classes in housewifery, maternity clinics, and the like. The school lunch program aims at democratization of better food supply and it has been most effective in thousands of communities. To the extent of one meal a day it reaches children of all families of the town. In spite, however, of wide distribution of federal funds the unprogressive community often fails to adopt the program or enacts it on a too meager basis.

As a measure of our progress in applied nutrition one may fairly look at the extent of conquest of the recognized deficiency diseases. Suitable fortification or enrichment of cheap staple foods seems to offer a partial remedy against unfavorable selectivity to the extent of supplying those specific nutrients which are economically and technically available through such a channel. Especially in hard times poorer people eat more cereals because they are the cheapest foods. Also, fortification is incomparably cheaper, when done on an industry wide basis, than any major revision of a family menu. Enrichment of cereals seems well fitted to reach the neediest. However, the filtering effect is not absent so long as it remains on a voluntary basis. The miller and the baker are eager to enrich the brands which they produce for the well-to-do trade but more reluctant to extend it to the cheapest brands which sell on a highly competitive basis to poorer and less informed consumers. Happily, many leading firms have adopted enrichment as a universal practice but many little mills and bakeries are not easily reached effectively. Compulsory enrichment, when enforced, meets this situation largely.

Despite the position of pellagra as our most conspicuous deficiency disease and of the well known association of corn with it, corn enrichment is only beginning. Much of the degerminated corn products are now widely enriched. This is far less true of whole corn meal which probably enters even more largely into the diet of southern people. The relative proportions are not precisely known. Whole corn meal is produced in about seven or eight thousand mills in the South, most of them tiny scattered operations. A concerted effort is required to develop a public consciousness which can be effective in such an industry. Legislation can neither be adopted nor enforced without the endorsement of strong public opinion. A good beginning has been made in South Carolina where nearly one fourth of the state's eight hundred mills are now voluntarily enriching their product. The movement is under way in other southern states and must eventually succeed.

Meanwhile there is much skepticism about the virtues of synthetic vitamins which have indeed suffered at times from excessive ballyhoo. We are being called on for evidence that public health is benefiting from these measures. The results must still be appraised inferentially rather than by the direct measure of vital statistics. This is also true of every other measure of mass nutritional betterment above mentioned. We need not therefore doubt their usefulness. In each case we must assure ourselves of the rationality and feasibility of the measure, then apply it conscientiously and leave the verdict to be rendered in the future. This requires patience, common sense, and genuine statesmanship as well as scientific knowledge.

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PRESENT KNOWLEDGE OF FAT IN NUTRITION

Fat is an important human dietary component both because of its high fuel value and the palatability that natural fats impart to food. Since the fatty acid molecule is nearly free of partial oxidation, fat yields over twice the caloric value of carbohydrate or protein and becomes the principal and most efficient form of energy storage in the body.

The optimal amount of fat in the diet cannot be stated with any exactness; certainly wide variations are compatible with health. The "Recommended Dietary Allowances" state that fat allowances must be based

more on food habits than on physiologic requirements, but that 20 to 25 per cent of the calories derived from fat are desirable.

Studies by R. E. Johnson and R. M. Kark (*Science* **105,** *378* (*1947*)) indicate that among American soldiers at hard work and even in different climates, a free choice of foods resulted in 33 to 40 per cent of the total calories (3200 to 4400 caloric total) being derived from fat.

Before discussing certain minimum and maximum limitations in the adjustment to dietary fat, there are also some recognized principles and effects concerned with the wide range of normal intakes. Of the three major nutrients (carbohydrate, fat, and protein), fat has the lowest specific dynamic effect and can confer economy of utilization upon the combinations in which it is present (*Nutrition Reviews* **3,** *82* (*1945*); **5,** *100* (*1947*)). This appears to be the principal basis for the observation of several workers that the "isocaloric" replacement of carbohydrate or protein by fat results in better growth. The effect is sufficiently great that the economy of using low fat rations for livestock feeding might be questioned or further investigated. Considerable gains have been noted in butterfat and milk production by increasing the fat in the ration of the dairy cow, although conflicting reports (see *Ibid.* **2,** *213* (*1944*)) indicate the need for further study of the type of fat employed and the total calories supplied. B. T. Scheer, S. Dorst, J. F. Codie, and D. F. Soule (*Ibid.* **5,** *272* (*1947*)) claim that physical capacity (swimming) of rats may be increased by increasing their dietary fat.

There have been many reports on the relative digestibility and absorption of different types of fats. In general, the more unsaturated fatty acids are better absorbed than saturated ones, but one type may influence another, and the nature of the glyceride structure in which a given fatty acid occurs appears also to affect its digestibility (*Nutrition Reviews* **3,** *278* (*1945*); **5,** *155* (*1947*)). Although earlier investigated with the opposite conclusion, later work on the fatty acids of butter (*Ibid.* **3,** *271* (*1945*)) indicates that the more unsaturated, liquid, fatty acids provide better growth and lead to greater storage of carotene than the saturated, solid fatty acids. Saturated fatty acids in the diet lead to greater deposition of fat in the liver than unsaturated fatty acids, hence the former may be contraindicated in hepatic disorders where there is a tendency toward fatty livers. This contraindication cannot necessarily be extended to the normal individual (*Ibid.* **1,** *133* (*1943*)). The possible superiority of butterfat over vegetable fat has been adequately discussed in previous reviews (*Ibid.* **2,** *267* (*1944*); **4,** *140* (*1946*)). It may be added only that J. Boer, B. C. P. Jansen, and A. Kentie (*Nature* **158,** *201* (*1946*)) have traced the growth promoting factor of "summer butter" to vaccenic acid (Δ-11,12 elaidic acid), and that R. P. Geyer and co-workers (*J. Biol. Chem.* **169,** *227* (*1947*)) have analyzed various fats and oils for their vaccenic acid content. Butterfat contained the most (0.5 to 0.7 per cent), lard, mutton fat, and beef fat were fair sources, while various vegetable oils contained none by the method of analysis employed.

In approaching the lower limits of fat in the diet, it must be recalled that food fats are the natural carriers of some of the vitamins, sterols, and other fat soluble compounds. A limitation of dietary fat might therefore limit the intake of vitamin D, tocopherols, and vitamin A. Such effects have appeared most prominently however when fat absorption is markedly retarded in various disease conditions. Poor absorption of the fat soluble vitamins occurs in sprue, pancreatic cystic fibrosis, celiac disease, and in general in conditions causing steatorrhea. W. J. Darby, M. E. Cherrington, and J. M. Ruffin (*Proc. Soc. Exp. Biol. Med.* **63,** *310* (*1946*)) have noted low plasma tocopherol levels in sprue and a poor plasma response to a test

dose. In normal animals however it has been difficult to prove that the fat content of the diet greatly affects, for example, the absorption of vitamin A (*Nutrition Reviews* **1,** *303* (*1943*)).

A rigorous exclusion of fat from the diet is required to produce the symptoms of "essential fatty acid" deficiency. Rats maintained on a fat free diet do not grow or maintain their weight, and develop skin disorders and kidney degeneration. This condition cannot be cured by feeding saturated fatty acids, but only upon administration of linoleic or arachidonic acids. These are highly unsaturated fatty acids and are components of phospholipids. It is probable that the same deficiency condition has been demonstrated in dogs (A. E. Hansen and H. F. Wiese, *Proc. Soc. Exp. Biol. Med.* **52,** *205* (*1943*)). In man, one subject has been placed experimentally for a long period of time on a fat free diet (W. R. Brown, A. E. Hansen, G. O. Burr, and I. McQuarrie, *J. Nutrition* **16,** *511* (*1938*)). A deficiency condition did not develop in six months, although the iodine number and linoleic acid content of the plasma fatty acids were decreased. An eczematous condition which develops in infants maintained on diets with very low fat content may be related to essential fatty acid deficiency (G. O. Burr, *Federation Proceedings* **1,** *224* (*1942*)).

Unless other fat soluble vitamins are fed with an experimental low fat diet, the picture may be complicated by other deficiencies. To what extent some of the newer fat soluble factors, for example vitamin E, may be concerned in the picture of essential fatty acid deficiency, is not known. Since vitamin E may function as a physiologic antioxidant for unsaturated fatty acids, relations between essential fatty acids and the tocopherols are predictable.

Turning to the effect of high fat diets, it is found that man and animals may tolerate large amounts of fat. Sudden shifts to a high fat diet may cause diarrhea and regurgitation due to delayed absorption and the formation of soaps, but a tolerance is soon developed. Excessive fat in the diet of dogs is said to cause a hemolytic anemia (*Nutrition Reviews* **4,** *83* (*1946*)), and in the diet of the cotton rat to cause an increase in tooth decay (*Ibid.* **4,** *308* (*1946*)). A natural limitation of the dietary fat lies in the metabolic disturbance, ketosis. The complete oxidation of fat, without formation of "ketone bodies," depends on a balance with carbohydrate oxidation. In an earlier concept, termed ketolysis, it was thought that there was a conjugation of acetoacetic acid and a carbohydrate derivative, upon which both were oxidized. This more strictly quantitative picture has been largely replaced by the Antiketogenesis concept, which implies that carbohydrates are preferentially oxidized, and that ketone bodies only accumulate when their rate of formation exceeds their oxidation by peripheral tissues. More recently, studies on the tissue level show that the metabolism of acetoacetic acid depends on condensation with oxalacetate (a carbohydrate derivative), to form members of the tricarboxylic acid cycle (*Ibid.* **4,** *23*, *207* (*1946*)). In a sense, this supports the earlier theory of ketolysis. The extreme example of ketosis is of course in diabetes where no doubt both the excessive catabolism of fat and the lack of carbohydrate oxidation products are responsible.

Rapid advances in knowledge of both the formation of fat from carbohydrate and the oxidation of fatty acids have been made, although many of the reaction details are lacking. Contrary to earlier beliefs that only gross excesses of carbohydrate are converted to fat and stored, isotope studies of the exchange of fatty acids in fats indicate that the conversion of carbohydrate to fat is proceeding continuously. The isotope experiments of D. Rittenberg and K. Bloch (*Nutrition Reviews* **4,** *295* (*1946*)) prove that acetate is a direct precursor of fatty acids, and other studies make it probable that acetoacetate may represent a first stage in synthesis, but higher intermediates or fur-

ther details of the process are obscure. Whether or not acetoacetate might undergo further condensations with acetate is not known, but it does not seem likely from the evidence on hand that acetoacetate is reduced to butyrate prior to further condensation, since butyric acid or tributyrin do not appear to be converted into higher fats (D. Rittenberg, R. Schoenheimer, and E. A. Evans, *J. Biol. Chem.* **120,** *503 (1937)*; M. G. Morehouse, *Ibid.* **155,** *33 (1944)*).

Although several of the vitamins, particularly thiamine, have been implicated in the process of fat synthesis from carbohydrate, none of these seems to be specific. Lack of these vitamins may cause decreased fat synthesis only through the diminished food intake common to several vitamin deficiency conditions (see *Nutrition Reviews* **5,** *191 (1947)*). In diabetic animals the experiments of DeW. Stetten and G. E. Boxer (*Ibid.* **3,** *213 (1945)*) indicate that the formation of fat from carbohydrate may be reduced to 5 per cent of normal. In the light of newer knowledge of the action of insulin, this may also be due to lack of carbohydrate intermediates, although a direct action on the synthetic processes of fat formation is not excluded.

Whether or not fat can be converted into carbohydrate has been a long-standing controversy, and it has been reviewed by H. J. Deuel and M. G. Morehouse (*Advances in Carbohydrate Chemistry* **II,** *120 (1946)*). Actually, the question is becoming a little academic since it is now recognized that fat and carbohydrate enter the same metabolic channel (tricarboxylic acids). With a given energy need, one may then "spare" the other.

As to the mechanism of the degradation of fatty acids, the experiments of S. Weinhouse, G. Medes, and N. F. Floyd (*J. Biol. Chem.* **155,** *143 (1944)*) with liver slices and octanoic acid containing isotopic carboxyl-carbon, strongly favor the theory that fatty acids are degraded 2 carbons at a time with random resynthesis into 4 carbon compounds. Since some deviations from the theoretical isotope analysis required for this mechanism have been noted, both in the experiments cited and by S. Gurin and co-workers (*unpublished presentation at Chicago meeting of Am. Soc. Biol. Chem. (1947)*), other pathways are not entirely excluded.

It has become possible to demonstrate the oxidation of fatty acids in tissue homogenates with the appropriate addition of catalytic factors (*Nutrition Reviews* **4,** *207 (1946)*). Among these factors are adenosine triphosphate, magnesium, and cytochrome-c. It seems probable that the fatty acid or some intermediate is phosphorylated during metabolism.

The metabolism of acetoacetate appears from other studies (F. L. Breusch, *Science* **97,** *490 (1943)*; J. M. Buchanan, W. Sakami, S. Gurin, and D. W. Wilson, *J. Biol. Chem.* **159,** *695 (1945)*) to consist of a condensation with oxalacetate to form members of the tricarboxylic acid cycle. Both carbohydrate and fat therefore enter a common channel of metabolism.

OCCURRENCE OF TUMORS IN PROLONGED CHOLINE DEFICIENCY

Various phases of the relation of choline deficiency to fatty livers and to liver cirrhosis have been discussed (*Nutrition Reviews* **1,** *88, 168 (1943)*; **2,** *180, 302 (1944)*; **3,** *143, 239 (1945)*; **4,** *246, 276, 345 (1946)*). Definite clinical improvement was reported in 7 of 10 patients with Laennec's cirrhosis when they were given up to 6 g. of choline daily (*Ibid.* **3,** *158 (1945)*). Enlarged livers decreased in size, ascites decreased or disappeared, serum proteins returned from hypoproteinemic to normal levels, and liver function tests became normal. Several other successful clinical treatments of liver

cirrhosis with choline alone or together with cystine have been described (*Ibid.* **4,** *180 (1946)*). However no therapeutic virtue of choline was observed in the treatment of 16 cases of infectious hepatitis when 1.5 g. of choline chloride were given daily as a supplement to a low fat, high carbohydrate diet (*Ibid.* **4,** *127 (1946)*). These observations seem somewhat promising yet do not prove that liver cirrhosis in man is partially a result of choline deficiency nor that high levels of choline are necessarily beneficial in producing a remission in the manifestations of liver cirrhosis.

L. L. Ashburn, K. M. Endicott, F. S. Daft, and R. D. Lillie (*Am. J. Path.* **23,** *159 (1947)*) raised the question of whether the stages in the development of liver cirrhosis in choline deficient rats are similar to those in liver cirrhosis in man. In a series of choline deficient rats, these authors injected either the hepatic vein or the portal vein with an injection mass which would differentiate the vessels of the hepatic vein system from those of the portal system. When the hepatic vein of choline deficient rats with cirrhotic livers was injected, the injected veins were regularly included in and connected by the trabeculae. On the contrary when the portal vein was injected, the injected veins were usually not in or connected by the trabeculae. The early depositon of fat was most marked about the hepatic venules. Ceroid soon appeared in the liver cells and groups of phagocytes collected about the hepatic veins and venules. These phagocytes gradually became incorporated into loose, fibrous trabeculae which formed not only around the central vein but also along the sublobular and large branches of the hepatic vein. In some experimental subjects, the first increase of connective tissue appeared about the large vessels, in particular near the hilar portion of the lobes.

In similar experiments where liver cirrhosis was produced by the injection of 0.05 ml. of carbon tetrachloride per 100 g. of body weight twice per week, fatty degeneration and necrosis occurred around the hepatic veins, mainly along the centrolobular branches. The trabeculae of fibrous tissue formed in essentially the same location as in choline cirrhosis. Thus the cirrhotic changes in both choline deficiency and carbon tetrachloride poisoning were definitely not portal in distribution but began and developed along the vessels of the hepatic system. In Laennec's cirrhosis the distribution of connective tissue proliferation observed in early cases has been around the vessels of the portal circulatory system. In this way the human liver cirrhosis would appear to have developed in a manner different from that produced by nutritional deficiency or by poisoning in experimental animals. On this basis the reasons for successful clinical treatment of cirrhosis with choline are not determined readily.

Typical fatty livers and liver cirrhosis were observed in rats in the early stages of experiments on the effects of prolonged choline deficiency by D. H. Copeland and W. D. Salmon (*Am. J. Path.* **22,** *1059 (1946)*). The diet in these studies consisted chiefly of alcohol-extracted peanut meal, alcohol-extracted casein, sucrose, lard, and a salt mixture plus the required water soluble and fat soluble vitamins. The rats were placed on experiment at 23 days of age and given a predetermined minimum amount of choline during the first two weeks to prevent death in acute choline deficiency. In cases of severe weight decreases after discontinuation of choline an occasional minimum dose was given when indicated to alleviate the acute deficiency signs. Fourteen of the 69 experimental rats died during the first six weeks; the remainder made moderate growth gains and were in fairly good condition for several months before they became unthrifty in appearance with a marked loss of hair, muscular weakness, drowsiness, and lethargy. All animals living eight months or longer had liver cirrhosis, many with the characteristic "hobnail" appearance with

nodules and pedunculated projections. In 75 per cent of the cirrhotic livers there was a globular substance throughout the interlobular connective tissue and in some cases in the lobules themselves. This substance was thought to be identical with the ceroid pigment described by R. D. Lillie, F. S. Daft, and W. H. Sebrell (*Pub. Health Reports* **56,** *1255 (1941); Nutrition Reviews* **4,** *137 (1946)*). Cysts which appeared to be agglomerates of detailed sinusoids were observed in 50 per cent of the cirrhotic livers. An extensive bile duct proliferation was observed in all the livers.

Neoplasms in the liver of the nature of adenocarcinomas were observed in 15 of the 50 choline deficient rats which survived eight months or longer. These neoplasms consisted of cords of cells, solid masses, and acini-like whorls of stratified cells. The epithelial cells were unlike bile duct proliferation since they were definitely stratified and not in simple acini. These growths were not necessarily at the periphery of the liver and frequently were massive, compressing the liver tissue. They appeared at times to arise from the liver cells themselves, and at other times from the center of the lobule or in the interlobular spaces. Hemangioendotheliomas up to 5 cm. in diameter were observed in the peritoneal and subcutaneous fat of 10 per cent of these rats. The lungs of 30 per cent of the rats showed nodules in various parts of the lungs or at times involving all parts. Sections of small pulmonary nodules indicated that they consisted of anaplastic oval cells of various sizes and acini-like structures of an adenomatous nature but the larger masses were more like primary medullary carcinoma. The epithelium lining bronchiectatic cavities exhibited marked metaplasia. Of the 50 rats with cirrhotic livers, 3 developed retroperitoneal growths of the sarcoma or fibrosarcoma type which through pressure on nerves produced paralysis of the posterior limbs.

These studies indicate the great difference which can occur in a specific dietary deficiency depending on its severity and duration. The demonstration of the widespread occurrence of neoplastic growths in prolonged choline deficiency may be the beginning of a different phase in tumor research.

One cannot help wondering what constitutional differences exist between the rats which developed neoplasms and those that did not, how soon these tumors could be diagnosed through some exploratory operative biopsy procedure, and if their growth in early stages could be arrested by an optimum choline regimen. Our present knowledge of the role of choline in the normal physiology of tissues is sufficiently meager that there is no apparent explanation for these aberrations in the proper interrelations of growth and development of the various tissues.

POSTOPERATIVE NUTRITIONAL REQUIREMENTS IN MAN

During the war much effort was expended to understand the nutritional requirements of wounded, postoperative, and burned patients during convalescence. Much of this work has already been reviewed (*Nutrition Reviews* **3,** *15, 40, 59, 222, 253 (1945);* **5,** *29, 46 (1947)*). C. Riegel *et al.* (*J. Clin. Invest.* **26,** *18 (1947)*) have added to the knowledge of the nutritional requirements for a short postoperative period. Fifty-five patients were studied: 29 after gastric operations, 20 after craniotomy or similar surgery, and 6 after miscellaneous operations. Nitrogen balance and plasma protein concentrations were followed for periods of five or ten days postoperatively. Included in the nitrogen excretion were urine, feces in most cases, wound drainage, and vomitus. In only 4 cases was the nitrogen loss from the drainage significant, and in no instance

did the vomitus contribute more than a trace to the nitrogen output.

Food intake was one of four kinds: the hospital diet prepared in a metabolic kitchen, protein hydrolysate ("Amigen"), lactalbumin hydrolysate, or a gastrostomy formula. Intake of calories in each case averaged from 2 to 46 per kilogram per day, with 12 cases receiving less than 20. Protein intake averaged from 0.19 to 3.9 g. per kilogram per day. Twenty-six cases had an intake less than 1.5 g. of protein per kilogram per day, and 29 had greater.

Thirty-seven of the 55 patients were in negative nitrogen balance, while 5 were in equilibrium and 13 in positive nitrogen balance. In general, those cases with nitrogen equilibrium and positive balance are those with intakes of 30 calories and 1.9 g. of protein per kilogram per day, or more. There was no significant difference between those with gastric operations and cranial surgery; nor was a correlation between age and sex found.

The authors found no correlation between the food intake or nitrogen balance and the plasma protein concentration, which was apparently measured by the difference between total plasma nitrogen and nonprotein nitrogen. The lack of correlation is not surprising since separation of albumin and globulin was apparently not made and it is generally recognized that acute illnesses are followed in time by a reduction in the plasma albumin concentration while the globulin concentration may increase, making the changes in total protein concentration insignificant.

The authors concluded that although there are great individual variations, surgical patients require a larger protein intake to prevent negative balance than do normal individuals. Unfortunately, however, the protein intake and calories were in rough proportion from patient to patient so that it is difficult, if not impossible, to separate the two variables. The caloric intake is as important or more so than that of protein, because of the well known sparing action of adequate calories upon protein catabolism. This is illustrated in 3 of the patients who had high protein intake (2.5, 3.7, and 3.8 g. per kilogram per day) and yet were in negative nitrogen balance presumably because the calories in these 3 patients were low (21, 16, and 11 calories per kilogram per day).

The fact that nitrogen equilibrium and positive nitrogen balance, in some cases marked, could be achieved in the first few postoperative days is important information. Others working on the same or similar problems have found such an achievement difficult, if not impossible, in well nourished individuals, but somewhat easier in individuals who had lost considerable weight before the operation, or who had other trauma. One would, therefore, like to have known the nutritional status of the individuals at the time of operation. It may be assumed, however, that many of the gastric cases were undernourished, making positive nitrogen balance easier to achieve.

No mention is made by the authors of blood, plasma, or albumin administration. It may be assumed that most of the patients received at least blood transfusions. The problem of how to enter into the nitrogen balance such whole protein is still controversial, and it would be of interest to know how the authors calculated it.

It should be noted that the authors used considerable hydrolyzed protein by mouth and found it as effective as whole protein in maintaining nitrogen balance. One wonders if the converse was not also true, that whole protein was as effective as hydrolyzed protein. If this were so, one would prefer to use whole protein because of its low cost and greater palatability.

VITAMINS A AND E AND MILK PRODUCTION

The total vitamin A potency of cow's milk and of butter depends upon the carotene content of dairy rations (*Nutrition Reviews* **2,** *70, 203 (1944)*). The level of true vitamin A in milk may be increased by feeding large amounts (700,000 units daily) of the vitamin in the form of fish liver oil concentrates although the gain is partially offset by a decrease in the carotene content (*Ibid.* **1,** *145 (1943)*). Supplementation of the diet of the cow with concentrates of natural, mixed tocopherols has little effect on the vitamin A content of milk but does increase the milk fat and total milk production (P. L. Harris, W. J. Swanson, and K. C. D. Hickman, *J. Nutrition* **33,** *411 (1947)*). These studies confirm the observation of H. J. Deuel and co-workers (*Ibid.* **22,** *303 (1941)*; **23,** *567 (1942)*) that administration of concentrates of vitamin A raises the level of true vitamin A and lowers that of carotene in cow's milk. The same results followed the simultaneous feeding of both vitamin A and tocopherols except that the fall in the carotene content of the milk was less marked than after supplementation with vitamin A alone. However, the increase in production of butterfat which followed administration of vitamin E did not occur if both vitamins A and E were supplied.

The experiments of Harris *et al.* were conducted over a twenty month period on a herd of 75 registered Brown Swiss cows. Similar results were obtained in a shorter study of a Guernsey herd. The cows were grouped according to age, stage of lactation, and relative milk production as determined from previous individual records. The herd was stall-fed on grain, corn silage, and field-cured hay except from May through August. Grain was supplied throughout the summer grazing period. The food during stall-feeding was estimated to supply an average of 550,000 units of vitamin A daily. Experimental supplements of vitamins A and E were specially manufactured powdered concentrates which were stabilized against destructive oxidation. These were spread on the grain mixture each day and were consumed completely. Individual records of butterfat and of total milk production were kept and vitamin A and carotene analyses were made twice monthly on pooled samples of milk.

Supplementation of the ration with 1 g. of natural, mixed tocopherols increased milk-fat concentration from 10 to 40 per cent and total milk production from 5 to 15 per cent. The actual volume of milk was slightly reduced but the over-all change in production on the basis of "4 per cent milk" was almost invariably an increase. The effect of the tocopherols was evident during the months of pasture feeding as well as during stall-feeding. Carotene in green foodstuffs did not interfere with the stimulating action of tocopherol although this was nullified completely by vitamin A *per se*. Changes in the fat concentration of the milk appeared within ten days after the addition or omission of vitamin E. The effect appeared independent of the concentration of the individual tocopherols in the supplement.

The efficiency of transformation of carotene into vitamin A was not improved by tocopherols and varied from 6 per cent of the intake of carotene during stall-feeding to less than 1 per cent for cows with a much higher carotene intake as a result of pasture-feeding. The total vitamin A content (true vitamin A plus carotene) of milk during stall-feeding amounted to 800 to 900 units per quart which was one half of the level found for cows eating green forage. Carotene contributed 25 and 40 per cent of the vitamin A potency in the winter and summer milks, respectively. As in the studies of Deuel *et al.* (*loc. cit.*) 700,000 units or more of vitamin A were required as daily supplements in order to raise the total vitamin A value of winter to that of summer milk.

Blood analyses were limited to single de-

terminations of tocopherol. Average values for the tocopherol-supplemented and the control groups were 1.06 and 0.45 mg. per cent, respectively. The level in the plasma of normal men is 1.0 mg. per cent, or more (M. L. Quaife and P. L. Harris, *J. Biol. Chem.* **156,** *499 (1944)*). The possibility was suggested that the higher value in the vitamin E-fed cows represented a normal level and that the blood of the control cows contains a subnormal quantity. Very little of the administered tocopherol was secreted in the milk. Summer and winter milk fat from control cows contained 42 and 23 micrograms per gram, respectively. These levels were raised to 51 and 47 micrograms by the feeding of 1 g. of tocopherols daily. The efficiency of utilization of the ingested vitamin E was no more than 2 per cent.

Of interest is the observation that milk from vitamin E-fed cows coagulated less quickly than milk from control cows whereas that from vitamin A-fed cows coagulated more quickly. The variations in the rate of curd formation could not be explained on the basis of differences in titratable acidity.

General improvement in appearance, reproduction, and health was noted following supplementation with tocopherols. Appetite was increased and generalized infections were absent. On only one occasion was it necessary to remove the fetal membrane following parturition. The record for the previous year, without vitamin E-supplementation, showed three stillbirths, four abortions, and a number of retained placentas requiring services of a veterinarian after the birth of almost every calf. These observations obviously need confirmation by more detailed studies before they can be accepted as the specific result of the administration of vitamin E.

The explanation of the effects of tocopherols in cows is obscure and is apparently different from the recently reported stimulation of milk output by iodinated protein (*Nutrition Reviews* **5,** *92 (1947)*). The authors suggest that the improvement of the herd and the increase in milk production were due in part, at least, to the antioxidant action of tocopherols in the rumen with resulting changes in the proportions of the various microorganisms. This is not impossible in view of the importance of bacteria in the nutrition of ruminants. The obliteration of the effect of tocopherol by simultaneous feeding of a concentrate of vitamin A is difficult to understand. There was little indication in these studies of the characteristic sparing action of vitamin E on vitamin A. It would indeed be surprising if the herds used in these experiments were deficient in vitamin E. If this finding is substantiated, the subjective evidences of improvement in vigor and in metabolic efficiency would be highly significant for the dairy industry. The studies of Harris *et al.* emphasize the paucity of detailed information on the real function of vitamin E in the organism. Current research suggests that the tocopherols may have far more dietary importance than is indicated by the textbook term, "antisterility vitamin."

RELATIONSHIP OF PANTOTHENIC ACID TO ADRENAL FUNCTION

Hemorrhage and necrosis in the adrenal cortices of rats fed diets deficient in pantothenic acid have been noted in many laboratories. Further cytologic and anatomic observations of the results of deficiency in this vitamin in rats have been made by H. W. Deane and J. M. McKibbin (*Endocrinology* **38,** *385 (1946)*). Thirty-two male albino rats of about 30 days of age were distributed in two groups. One group (13 animals) received the control ration ad libitum. The ration contained 72 per cent

sucrose, 18 per cent casein, salts, corn oil, cod liver oil, choline, riboflavin, pantothenic acid, thiamine, pyridoxine, and niacin. The second group (19 animals) received the same ration with pantothenic acid omitted. At weekly intervals, for six weeks, 2 animals of the control group and 3 animals of the deficient group were killed for study.

Good growth (*ca.* 3 g. per day per rat) occurred on the control ration during the experimental period, whereas markedly impaired growth occurred in the deficient animals. Incidental observations showed the size of the thymus gland in proportion to body weight was much smaller in the deficient animals than in the normal animals at each of six observation periods. The relative weights of the adrenals of the control rats decreased with age as previously reported (V. Korenchevsky, *J. Path. Bact.* **54,** *13 (1942)*); but those of the deficient animals remained high throughout the experimental period. No significant differences in sugar or nonprotein nitrogen levels of the blood were observed in the two groups.

Histologically, changes in the adrenal cortex rapidly became evident, with gradual loss of sudanophil granules from the zona fasciculata with eventual complete loss of lipid droplets from the fasciculata, and a reduction in lipid content in the more peripheral zones as well. Of greatest interest was the rapid disappearance in cortices of the deficient animals of all ketosteroid reactions, as evidenced by the fact that tests for ketosteroids were entirely negative in the reticularis and inner fasciculata and only faintly positive in the outer fasciculata by the end of only one week. Complete exhaustion of the ketosteroid content of the cortex occurred in six weeks. The severely deficient animals also exhibited a decrease in liver glycogen, with some shrunken and often dying liver cells.

These symptoms are interpreted as indications that lack of pantothenic acid acts as an "alarming agent" for the rat. Such agents cause the release of pituitary adrenotropin which stimulates the adrenal to enlarge and increase its secretion of corticosterone. The severity of the reaction in this vitamin deficiency is apparently such as to cause complete adrenal exhaustion of corticosterone.

A second connection between pantothenic acid and adrenal function is afforded by results of E. P. Ralli (*Endocrinology* **39,** *225 (1946)*). Thirty-five day-old rats were placed on a ration deficient in pantothenic acid; after thirty to thirty-five days on this diet, the adrenal glands were removed and the rats divided into groups, which in addition to the deficient ration received (1) water containing 1 per cent sodium chloride, (2) water + 1 per cent sodium chloride + desoxycorticosterone acetate (0.5 mg. per day, injected), (3) water + 1 per cent sodium chloride + cortin (0.4 ml. per day), and (4) water + 1 per cent sodium chloride + calcium pantothenate (40 mg. per 100 g. of ration). In groups 1, 2, and 3, 50 per cent or fewer of the animals survived as long as ten days; none survived seventy-five days. Survival in the hormone supplemented group was only slightly better than in group 1. In the group receiving pantothenic acid, all of the animals were alive at the end of ten days; 67 per cent were alive at seventy-five days, and 7 per cent (5 of 70) were still alive at three hundred days. When salt was eliminated from the drinking water, but the groups were otherwise the same, survival was sharply limited, and only the animals receiving desoxycorticosterone acetate were benefited. Under these conditions, animals of group 2 survived on the average eleven and one-half days; those of groups 1, 3, and 4 averaged only five and three-tenths to six and two-tenths days of survival. In these experiments all animals except those of group 4 were subjected to the double strain of pantothenic acid deficiency and adrenalectomy. No data are cited to show survival times of pantothenic acid deficient animals

which have not been adrenalectomized, and the favorable effect of pantothenic acid might well have resulted from its action at sites not closely concerned with the action of the adrenals. It is clear from work of others, however, that survival on the pantothenic acid deficient rations is curtailed considerably by adrenalectomy.

NUTRITIVE VALUE OF PROTEINS IN MIXED DIETS FOR DOGS, RATS, AND MAN

While it is known in general that the supplementation of vegetable diets with a high quality protein such as meat causes an improvement in the nutritive value of the ration for all species which have been studied, the details have yet to be worked out. It seems that the essential amino acids for the rat, man, dog, and chick are similar but not the same. It is likely that the quantitative relationship of the essential amino acids may also vary among the species. If such differences exist, then various proteins must have different biologic values for different species. It is known that the relatively poor nutritive value of casein for the chick is due to its low arginine and glycine content. Little information of this character is available for other species.

D. M. Hegsted, A. G. Tsongas, D. B. Abbott, and F. J. Stare (*J. Lab. Clin. Med.* **31,** *261 (1946); Nutrition Reviews* **4,** *264 (1946)*) reported on the biologic value and digestibility of proteins in man. In a later report (D. M. Hegsted, V. Kent, A. G. Tsongas, and F. J. Stare, *J. Lab. Clin. Med.* **32,** *403 (1947)*) a comparison is made of the nutritive value of proteins in diets for dogs, rats, and man. Biologic value was considered as representing the per cent of absorbed nitrogen retained in the body.

Digestibility was taken as the per cent of fed protein absorbed. It is pointed out that while the reasons for differences in the digestibility of proteins are obscure, specific amino acid linkages, proteinase inhibitors, amount of fiber in the food, method of preparation of the food or protein, and amount and kind of the various proteinases in the digestive apparatus are factors which may be involved. It is to be expected that not all factors would be of equal importance in different species. Thus a predominantly carnivorous animal with a short digestive tract might be expected to digest the proteins in vegetable matter less effectively than predominantly vegetarian species.

The diets used were similar to those in the earlier study in human subjects. The basic diet was an all-vegetable diet containing no animal protein, approximately 50 per cent of the protein in this diet coming from white bread made without milk solids. When supplements were added, each constituent of the diet was reduced by one-third and the amount of protein thus removed was replaced by an equivalent amount of protein from the supplements. The supplements were meat, soy flour, wheat germ, or bread. In general, the plan followed was to feed a nitrogen-free diet for a period of ten to twenty days, urine and feces being collected during the last week. The urine was combined to give composite samples representing a three and four day period. The feces were homogenized with water and combined in a similar manner, charcoal markers being used to delimit the feces into appropriate periods. Following the nitrogen-free period, supplements of the experimental diets were given. Generally the all-vegetable diet was fed for one week followed by a week during which the diet containing meat was used to supply a similar amount of nitrogen. This was followed by another period of the vegetable diet and subsequently other diets. There was considerable difficulty in completing satisfactory studies with dogs because the diets were apparently not palatable, at least after a depletion period.

The average results in the dogs indicated

an increase in digestibility and biologic value of approximately 10 per cent when meat was included in the diet. The standard deviations, however, were large for each value especially for biologic values. Striking differences were not obtained in adult rats, but in young growing rats all three supplements clearly improved the nutritive value of the diet, whether the diets were fed ad libitum or were restricted to the intake of the poorest animal. Meat, wheat germ, or soy flour supplements were not significantly different from one another.

In general, it appeared that the results on the rats and dogs were similar to those obtained with man, although there were some apparent differences. The most reliable data on all species were obtained with the all-vegetable diet and the digestibility of this diet was better in man than in the other two species. The biologic values, on the other hand, were in the same range for all species although somewhat lower for dogs. The net protein values suggested that man utilized the all-vegetable diet somewhat better than the other two species because of the more efficient digestion. Man and the rat appeared to react in nearly identical fashion to the addition of meat to the diet. Digestibility was not improved but the figures for biologic value were increased by approximately 10 per cent. The dogs, however, apparently digested the meat-supplemented diet better, in addition to showing an increase in biologic value with a resulting over-all net gain of 16 per cent as compared with 8 per cent. As to the soy flour-supplemented diet no improvement was noted with this supplement for human beings in either biologic value or digestibility but improvement was noted in the rats. The wheat germ supplement, while resulting in a gain in the net protein value of like amount in the two species showed improvement in man entirely in biologic value whereas it was greatest in digestibility for the rat. The observations on man, however, were too few to conclude that the apparent species differences in the response to these two proteins were real.

PYRIDOXINE DEFICIENCY AND NIACIN SYNTHESIS

The marked increase in the excretion of niacin and of N′-methylnicotinamide in the urine when dl- or l-tryptophane was fed, and other interrelationships between tryptophane and niacin metabolism have been reviewed (*Nutrition Reviews* **5,** *110 (1947)*). S. Lepkovsky and E. Nielsen (*J. Biol. Chem.* **144,** *135 (1942)*) and S. Lepkovsky, E. Roboz, and A. J. Haagen-Smit (*Ibid.* **149,** *195 (1943)*) showed that in rats and dogs maintained on a pyridoxine deficient ration, kynurenic acid (a normal metabolite of tryptophane) is replaced in the urine by xanthurenic acid. Since the normal metabolism of tryptophane in rats yields N′-methylnicotinamide in addition to kynurenic acid, F. Rosen, J. W. Huff, and W. A. Perlzweig (*J. Nutrition* **33,** *561 (1947)*) were interested in investigating the effect of a pyridoxine deficient ration upon the production of N′-methylnicotinamide. Pyridoxine deficiency was produced in 6 rats by feeding a ration relatively high in protein (35 per cent casein). Urinary xanthurenic acid excretion was measured quantitatively by the formation and determination of a green ferric salt in an alkaline medium. Urinary N′-methylnicotinamide excretion was measured by an acetone fluorometric method. As the pyridoxine deficiency became more intense as measured by the increased excretion of xanthurenic acid, there was a progressive decrease in the urinary excretion of N′-methylnicotinamide. When a dose of 100 mg. of dl-tryptophane was given after twenty-six days of pyridoxine depletion, the average excretion of N′-methylnicotinamide was only 177 micro-

grams in forty-eight hours. In contrast, 2 control rats after thirty-five days on the same diet supplemented with 2 mg. per cent of pyridoxine excreted 3420 and 1690 micrograms of N′-methylnicotinamide in the forty-eight hour period after the ingestion of the same supplement of tryptophane. To test if there had been an increased ability to destroy N′-methylnicotinamide during the pyridoxine deficiency, 50 mg. of N′-methylnicotinamide were given by stomach tube. A normal excretion was observed with a recovery of 16 to 28 per cent in the urine during the following twenty-four hours. In another series of rats ingestion of 25 mg. of niacin resulted in a normal excretion of N′-methylnicotinamide.

After the rats had been maintained on the pyridoxine deficient ration for thirty-one days, each rat was given 5 mg. of pyridoxine hydrochloride daily for four days. Xanthurenic acid disappeared abruptly from the urine but there was only a relatively small increase in the excretion of N′-methylnicotinamide. The administration of 50 micrograms of pyridoxine per day after the original massive supplement failed to produce any marked increase in the N′-methylnicotinamide excretion. During this period of pyridoxine therapy, there was a good growth response but the further supplementation of the ration with biotin and folic acid or of brewers' yeast and Wilson liver fraction L did not influence the excretion rate. Subcutaneous and oral administration of 5 mg. of pyridoxal daily proved as ineffective as pyridoxine in the above experiments.

From the above studies it would seem that there is a definite relationship between the maintenance of normal pyridoxine metabolism and the maintenance of the synthesis of niacin by the rat from tryptophane. The long delay in the return of this mechanism toward a normal range after the alleviation of the pyridoxine deficiency cannot be explained at the present time.

METABOLIC PROBLEMS AND MOLDS

Some of the interesting problems of metabolism presented by specific variations of the mold Neurospora have already been considered in this journal (*Nutrition Reviews* **4,** *326 (1946)*). An investigation by D. Bonner (*J. Biol. Chem.* **166,** *545 (1946)*) describes experiments which bear on the basic hypothesis employed in accounting for the experimental data; to wit, that there is a 1: 1 relation between chemical reaction and gene.

While a single enzyme may catalyze a number of reactions of a common type, general experience with mutants of Neurospora is that a single gene mutation results in the impairment or failure of only a single chemical reaction. An apparent exception to this generalization was reported by D. Bonner, E. L. Tatum, and G. W. Beadle (*Arch. Biochem.* **3,** *71 (1943)*) who found that strain 16117, which differs from the parent strain by a single gene, has a growth requirement for the two amino acids, isoleucine and valine. At that time this result was explained as a mutation affecting an enzyme able to act on more than one closely related compound, so that it was explicable if corresponding reactions leading to the biogenesis of the two amino acids were affected.

Subsequent work on this problem has elicited much new information, and has shown that the earlier hypothesis is untenable. This evidence was obtained by measuring the growth of the strain in the presence of isoleucine, valine, synthetic ketoisoleucine, and synthetic ketovaline singly and in various combinations. In addition to measurements with the strain 16117, requiring both valine and isoleucine in the nutrient medium, corresponding measurements were made with strain 33051 which requires the addi-

tion of valine but not isoleucine to the medium, and with an isoleucineless strain of Escherichia coli, no. 58-336, which requires isoleucine but not valine in its growth medium. From the actions of these three strains of microorganisms it was possible to unravel the events in strain 16117 of Neurospora.

With strain 16117 a certain ratio of concentrations of the two amino acids in the medium was required, the ratio giving maximum growth being 70 per cent 1(−) valine to 30 per cent 1(+) isoleucine. If to this optimum mixture either valine or isoleucine was added in excess, then complete growth inhibition occurred, which could be reversed by adding more isoleucine or valine respectively. As the author points out, these observations suggest that a genetic block of either isoleucine or valine synthesis would result in a double requirement. Externally supplied isoleucine would inhibit valine utilization in a mutant with a true isoleucine requirement, thus producing an apparent valine requirement; similarly a true valine requirement could produce an apparent isoleucine requirement. However, while this is almost certainly a contributing factor to the observed double requirement of strain 16117, it will not alone account for it, since strain 33051 will grow on valine alone. The mutual inhibitions described above for 16117 would not account for its double requirements, since evidence was found that similar inhibitions occur with strain 33051 although not of sufficient magnitude to prevent detection of single requirements.

With pure synthetic ketoacids, confirmation was obtained of the results previously observed with strain 16117 and impure preparations of the ketoacids from natural preparations (Bonner, Tatum, and Beadle, *loc. cit.*), namely that in the presence of isoleucine, ketovaline was active, but a mixture of ketovaline and ketoisoleucine was inactive. On the other hand, in similar circumstances, no activity of ketoisoleucine for the growth of 16117 was ever observed—a result which is regarded as highly significant and upon which the ultimate conclusions are largely based. Since the hydroxy acid analogue of valine can only be used by 16117 after a lag period of several days, while ketovaline is active (more active than valine itself at low concentrations) it was concluded that ketovaline acts as an intermediate in valine synthesis. In the same way, evidence for ketoisoleucine as an intermediate was obtained, but only by using isoleucineless E. coli. Ketoisoleucine was also active for another strain of Neurospora, but not for 16117 when tested in the presence of either valine or ketovaline. From this result it was concluded that strain 16117 is unable to perform the amination of ketoisoleucine, and the requirement of this strain for isoleucine is due to this inability to aminate ketoisoleucine. The corresponding transformation of ketovaline to valine was however demonstrated, as mentioned above.

Further investigations with the ketoamino acids confirmed the finding that strain 16117 is unable to convert ketoisoleucine to isoleucine, although it should perform this synthesis. Ketoisoleucine might therefore be expected to accumulate in the mycelium and then block the amination of ketovaline, since it has been found that the addition of the two ketoacids together does not support growth of 16117. Such an action of an accumulation of a precursor has been observed previously on several occasions, so the precedent has already been set.

It still remains possible therefore to account for the double growth requirement of strain 16117 on the basis of a mutant having a single gene change and a corresponding obligatory requirement of isoleucine. The second requirement of valine arises in secondary fashion because the failure of amination of ketoisoleucine leads to an accumulation of this ketoacid in the mycelium, and it has the power to inhibit the amination of ketovaline, making it necessary to supply valine in the medium.

DIETARY FAT AND PHYSICAL CAPACITY IN RATS

In most experimental studies, nutritional status of animals has been evaluated by use of criteria such as growth, reproductive activity, lactation, and gross pathology. During the recent war, several tests with human subjects were made to determine the effect of nutrition on "physical fitness," as judged by performance tests under standard work loads, etc. Comparatively few such tests have been made with experimental animals, where the various factors which influence physical performance can be more rigorously controlled.

B. T. Scheer, S. Dorst, J. F. Codie, and D. F. Soule (*Am. J. Physiol.* **149,** *194 (1947)*) cite results of such measurements with rats, as affected by variable levels of fat in the diet. As the criterion for physical capacity, a swimming test was used. In this test, as usually carried out, rats are loaded with a constant weight and allowed to swim to exhaustion. The duration of swim is taken as a measure of physical capacity. Using this test it was noted that animals with low specific gravity swam longer than animals from the same colony with a higher specific gravity. When the specific gravity of all test animals was adjusted to the same value (1.04 to 1.06) by hanging additional weights on the less dense animals, considerably better agreement was found between individual animals in the performance test. When the test was repeated with the same animals on successive days, a marked increase in duration of swim was noted, due to the training and skill acquired by the animal as a result of previous swims. This effect was eliminated by increasing at intervals of three minutes the weight carried by the animal during the swim. The time of exhaustion was taken as the point at which the animal was unable to reach the surface during ten seconds. With this method, tests with the same animals on successive days showed excellent agreement.

In preliminary experiments with the improved method, it was found (a) that duration of swim was positively correlated with body weight and with specific gravity of the animals; the method is completely satisfactory, therefore, only when applied to animals of similar size; (b) that there was no convincing difference in the physical capacity of male and female rats; (c) that young rats kept in large cages had greater physical capacity than similar animals kept in small cages; this difference disappeared as the animals became older, and was attributed to the fact that young rats in the large cages got more bodily exercise than those in small cages, but with age, both groups became more sedentary; (d) that physical capacity decreased progressively as caloric intake of the animals was lowered from nearly normal to a level just sufficient to maintain life during twelve weeks. A six week period of ad libitum feeding following the restricted intake restored the values to nearly normal levels.

In the chief series of experiments, weanling rats were fed diets which varied in fat content from 0 to 40 per cent but provided for a constant intake of protein, vitamins, and minerals. Though the diets were fed ad libitum, a regular and statistically significant increase in physical capacity was found as the fat content of the diet was increased. Control animals were fed sufficient methyl linolate to eliminate lack of essential fatty acids as a cause for the difference.

The authors raise the question whether the differences observed in physical capacity might not be due simply to differences in size of the animals, since growth was greater on diets high in fat than on those lacking in fat, since caloric restriction resulted also in marked retardation in growth, and since their preliminary studies showed positive correlation between physical capacity and weight. This question cannot be answered without further experiments designed specifically to settle this point.

FAILING VISION DUE TO NUTRITIONAL DEFICIENCY

From Jamaica in the British West Indies reports have come from time to time concerning a disorder characterized by increasing impairment in vision. Thus, H. H. Scott (*Ann. Trop. Med. Parasitol.* **12,** *109 (1918)*) described impaired vision and hearing together with itching and burning of the eyes, excessive sensitivity of the eyes to light, inflammatory lesions in the mouth, fissures, and salivation. In this report reference was made to an account of dimness of vision and impaired hearing associated with "peripheral neuritis" by H. H. Strachan in 1888 and 1897. L. M. Clark of Jamaica is quoted by Whitbourne (*see below*) as having reported in 1936 that a type of impaired vision associated with cracks and fissures of the lips was common in malnourished children between the ages of 8 and 14 years. His cases were noted following a food shortage on the island which developed after a severe hurricane. Nutritional edema was also present in these children. Usually the children did not complain but the parents or teachers noted that the child was not seeing well. The physician, however, was unable to improve the vision with glasses. Syphilis and foci of infection could be excluded as etiologic factors. Early in the disease the optic discs appeared normal but later pallor on the temporal side was often observed. The associated finding of peripheral neuritis and other evidences of faulty diet led to the use of cod liver oil plus a good diet containing milk, eggs, and vegetables with subsequent improvement. In some patients in whom treatment was discontinued recurrences were observed. Patients over 16 years of age were few and they did not respond well to the treatment. The latter was assumed to be due to atrophic changes which had already taken place and which could no longer be corrected by dietary management.

Similar cases are the subject of reports by D. Whitbourne (*Am. J. Ophth.* **30,** *169 (1947)*) and F. D. Carroll (*Ibid.* **30,** *172 (1947)*). The former refers to her observations on 74 cases treated at the School Clinic in Kingston during the year 1945. The children were all of mixed European and African blood or almost purely African. Girls outnumbered boys roughly in the ratio of 2:1 and the age range was 9 to 16 years with the majority 12 to 13 years of age. Curiously enough, all the patients except 2 came from urban areas despite the fact that rural children are usually more poorly nourished than town dwellers. The patients came from the poorer socio-economic groups in the majority of cases. Angular stomatitis and glossitis were often present and the skin was unusually dry and exhibited uneven pigmentation. In some cases, the skin was also thickened and cracked. Many children, however, appeared to be in fairly good condition. Symptoms were few because children and parents alike were usually unobservant. It was observed that the children could not see the blackboard or that the print seemed to run together. Occasionally unusual sensitivity to light was noted. Deafness was present as a serious concomitant sign in a number of these cases. Blind spots (scotomas) placed centrally in the visual field were discovered by Carroll in a number of instances and color vision was also impaired. In some cases the pallor of the optic discs described by earlier writers was noted also.

The children were treated with brewers' yeast and cod liver oil and in 50 of them satisfactory progress was noted. Among the remainder there was little improvement. Of these some came to attention late in the disease and in the others cooperation was poor. Improvement was first noted in the general condition, particularly in the mouth lesions. No case with impaired vision was cured without the oral lesions being healed at the same time.

A similar disorder has been described in Africa (D. F. Moore, *Lancet* **I,** *1225 (1937)*),

and in the recent war impairment of vision developing in association with symptoms and signs suggesting pellagra and wet beriberi has been described in former prisoners of the Japanese. Carroll examined some of these ex-prisoners and found blind spots in the retina in a position similar to that seen in the children in Jamaica and differing from the scotoma resulting from poisoning by tobacco or ethyl alcohol. H. Ridley (*Brit. J. Ophth.* **29,** *613* (*1945*)) noted in released prisoners of war a high proportion of double vision together with nerve deafness unassociated with other neurologic disorders. In the men examined by W. L. Roberts and T. H. Willcockson (*Am. J. Ophth.* **30,** *165* (*1947*)) temporal pallor of the optic discs and central scotoma like those seen in the Jamaican children were present. No improvement in vision had taken place even though high caloric, high vitamin diets had been fed for a considerable time. The original visual symptoms had developed in these cases two to three years previously, an observation which supports the opinion of D. F. Moore (*J. Trop. Med. Hyg.* **42,** *109* (*1939*)) that the likelihood of return of vision following improvement in diet is good only if the visual symptoms have been of less than six months' duration.

PERNICIOUS ANEMIA AND SYNTHETIC FOLIC ACID

When liver extract was first discovered two problems of importance arose; namely, whether its administration would effectively maintain the hematologic status and, secondly, whether neurologic disorders could be treated effectively by the use of concentrated liver extract or their development prevented. For some years there was considerable uncertainty on the latter point, some clinicians maintaining that crude liver extract was required to maintain the integrity of the nervous system whereas others claimed that liver extract even in concentrated form was adequate for this purpose. Time has shown that the latter concept is correct.

Synthetic folic acid has been available for about a year and reports are appearing which bear on these two questions. C. F. Vilter, R. W. Vilter, and T. D. Spies (*J. Lab. Clin. Med.* **32,** *262* (*1947*)) describe the results of treatment of 21 persons with pernicious anemia for one year. Seventeen persons treated with 10 to 15 mg. daily or 30 mg. three times a week given orally were maintained in apparent hematologic remission for one year. Three patients with pernicious anemia in whom treatment was begun during a relapse responded satisfactorily to a initial dose of 10 mg. of folic acid but 2 of them required increasing amounts daily to maintain the remission. The third patient received 50 mg. daily for most of the year in order to determine the effect of the drug on peripheral neuritis. Folic acid given orally controlled the manifestations of sprue in 2 persons for one year, nutritional macrocytic anemia in one person for seven months, and macrocytic anemia secondary to ileosigmoidostomy in one person as/or more effectively than liver extract.

However, folic acid in doses of 10 to 50 mg. daily did not prevent the development of degeneration of the posterior and lateral columns of the spinal cord in 4 persons with pernicious anemia. Only one patient of the entire series had subjective complaints or objective evidence of active central or peripheral nervous system degeneration prior to the administration of folic acid. This patient had numbness and tingling in the hands and feet, a stocking type of hypesthesia to touch and pin prick, and diminished knee and ankle reflexes interpreted as signs of peripheral neuritis. While receiving folic acid, 10 mg. daily, the signs of peripheral neuritis increased. Remission or progression in these signs could not be correlated with increases or decreases in the dosage of

folic acid and for the last nine months the patient's complaints and neurologic abnormalities had been essentially the same as when treatment was begun.

In 4 subjects with pernicious anemia numbness and tingling sensations in the hands and feet commenced gradually after five to eight months of folic acid therapy. In addition, unsteadiness of gait and moderate stiffness of the extremities developed. It is significant that these neurologic signs appeared after the hematologic status had reached or was near normal and gradually progressed until there was definite evidence of combined system disease. The neurologic abnormalities implicated the posterior columns and peripheral nerves in 4 of the subjects, the lateral columns in 3, and the cerebral cortex in one. When signs of nervous system degeneration were unequivocal, usually indicated by the appearance of a positive Romberg sign, the extensor response on plantar stimulation, loss of sense of position and of perception of vibration in the feet, and hypesthesia and hypalgesia in fingers and toes, each of these 4 patients received 50 to 500 mg. of folic acid orally per day for ten to forty days. Administration of these large doses of folic acid was followed by no subjective or objective improvement. Refined liver extract, on the other hand (10 U.S.P. units per milliliter), 3 to 5 ml. given intramuscularly per day, was begun after it was obvious that amelioration of the neurologic signs and symptoms would not occur or when progression of the disease was so rapid as to endanger the patient's future well-being. Within ten days thereafter, improvement in the neurologic status began and continued on somewhat reduced doses thereafter.

While the data presented are insufficient to make it clear whether folic acid in sufficient dosage will permanently maintain a hematologic remission in pernicious anemia, they certainly show that folic acid does not prevent the occurrence of changes in the nervous system nor does this substance even in massive doses halt or reverse the degenerative process once it has begun. In this respect it seems to be different from liver extract and the conclusion seems clear that, as many investigators have suspected, pernicious anemia represents a combination of at least two deficiencies, one manifesting itself in macrocytic anemia and the other resulting in degenerative changes in the nervous system. It follows that folic acid cannot be considered a satisfactory or complete treatment for pernicious anemia and should not replace potent liver extract in routine treatment until another method for the protection of the nervous system is devised.

UTILIZATION OF FAT ADMINISTERED INTRAVENOUSLY

Parenteral feeding in man is greatly handicapped by the difficulty of providing sufficient calories. The advantages of the only slight osmotic properties and the high caloric content per gram of emulsified fat make it an ideal supplement to glucose and protein hydrolysates if it can be shown to be utilized and emulsions suitable for use in man can be devised. L. J. Dunham and A. Brunschwig (*Nutrition Reviews* **3,** *302* (*1945*)) believed that the lard and olive oil emulsions they infused in dogs were not utilized.

J. M. McKibbin, R. M. Ferry, and F. J. Stare (*J. Clin. Invest.* **25,** *679* (*1946*)) gave daily infusions, in 4 dogs, of an emulsion of 150 g. of refined coconut oil, stabilized with purified soybean phosphatides and disodium phosphate, and with added cholesterol in most instances. The dogs were fed a basic purified diet of sugar, casein, "Amigen," coconut oil, salt mixture, and vitamins. In 3 of the dogs nitrogen balance was followed at intervals. Two dogs were killed at the end of the experiment and the carcass fat determined. By comparing the iodine and saponification numbers of the carcass fat with

that of the coconut oil infused, the maximum amount of remaining coconut oil could be calculated. In the 2 dogs this was 57 and 37 per cent, respectively, indicating, according to the authors, that the remaining infused fat was utilized (it was not excreted in urine or feces). As additional evidence of utilization of the infused fat, positive nitrogen balance was obtained in 3 of the dogs. The nitrogen balances were averaged for the period, the urine and fecal determinations having been done on pooled collections. In one study a negative nitrogen balance in a dog on a low caloric intake by mouth was followed by positive balance when the caloric deficiency was made up by intravenous fat emulsion. Body weight during the infusion periods was either maintained or increased, depending in part upon the previous state of nutrition of the animal. This appears to be good evidence that the fat infused was used by the dog.

Liver excretory function (bromsulfalein elimination) was impaired in the 2 dogs in which this determination was made, although prothrombin time remained normal. The bromsulfalein excretion returned to normal in one dog which was followed after the infusions for ten days on a milk diet.

It is noteworthy that 2 of the dogs apparently became ill and refused their food after prolonged infusions. No explanation for this is offered. Three of the dogs had complete postmortem examinations which revealed small granulomatous lesions in the lungs and spleen, and evidence of fat phagocytosis in the reticuloendothelial cells of the liver and spleen. There was no accumulation of fat in the parenchymal cells of the liver.

EFFECT OF WARTIME STARVATION IN HOLLAND UPON PREGNANCY AND INFANT HEALTH

Although it has long been noted that maternal malnutrition is associated with improper growth or development of the fetus, prematurity, stillbirth, and neonatal death, and even with toxemia during pregnancy, such observations have usually been made in persons in whom suboptimal health and hygiene have led to the use of improper diets. Circumstances in which maternal diet was the only variable have been comparatively few. The mass malnutrition in western Holland during the winter of 1944 and 1945 presented a situation in which, in the main, diet alone was the factor. A study made in Rotterdam and the Hague is presented by C. A. Smith (*Am. J. Obst. Gynec.* **53,** *599 (1947)*).

In Holland the food situation began to deteriorate in October 1944 and in December it reached a low level which was rather steadily maintained until liberation of the country in May 1945. Thus the critical period lasted at least five months but not more than eight. Pregnancies ending just before relief of hunger had begun in relatively good nutritional circumstances; on the other hand, those beginning during hunger were ended after relief. It is estimated that in September 1944 the total calories available to pregnant women were 1925, in February 1945 they were 731, and in April of that year 912. Fat was greatly reduced, and protein less so. The protein of the diet was somewhat altered, however, in that a much greater reduction in animal protein occurred than in that derived from vegetables.

Upon pregnancy in general an outstanding effect of hunger was a fall to one third of the expected number of births representing conceptions during the undernutrition period. The undernutrition period was dekram by cessation of menstruation in about 50 per cent of women, with normal menstrual periods reported in only about 30 per cent. It is interesting that menstruation was regularly resumed when food was again available.

Though fewer women conceived during the months of undernutrition, those who did so or who gave birth at that time were not essentially different from the patients of prehunger or posthunger times as indicated by the records of the National School for Midwives at Rotterdam and the obstetrical service of the Zuidwal Hospital at the Hague.

The statistics concerning toxemia of pregnancy are of interest. A number of German authors reported a decrease in toxemia during starvation in World War I (*J. Am. Med. Assn.* **68,** *732 (1917)*). Likewise the Dutch obstetricians reported less than the expected amount of toxemia during the hunger period of World War II. The analysis made by Smith also suggests that a reduction in incidence of toxemia occurred. This was so whether the incidence was based on the per cent of cases diagnosed as toxemia unqualifiedly, or when the diagnosis was restricted to those cases in which the blood pressure was at least 140 mm., albumin was present in the urine, edema was present, or convulsions occurred. There was a definite scarcity of table salt during the hunger period and the question is raised whether this may have been of some significance in relation to the lower incidence of this complication of pregnancy.

The effects of maternal nutrition upon the birth weights of infants born in Rotterdam and at the Hague were noteworthy. At the beginning of the critical period, birth weights were distributed over about the same range as in the previous winter. Following this, there was a decline in birth weight which was greater among the constitutionally larger infants than among the smaller ones, a fact which was equally notable in the rise after liberation and availability of food. Since the undernutrition of mothers giving birth just before the liberation must have begun much earlier in pregnancy, the data suggest that birth weight reflects the nutrition of late pregnancy rather than that earlier in gestation. Data concerning birth length were less conclusive than those dealing with birth weight but this is not unexpected since length is only one dimension while weight is the product of three.

The small number of births several months after improvement of the food situation, reflecting conception at the worst phase of starvation, revealed a reduction of fertility during the hunger period. No significant data for abortion or miscarriage could be obtained. During circumstances of marked nutritional inadequacy no significant increase in premature births was noted. Likewise there was no increase in incidence of stillbirth during the hunger winter. Neonatal death occurred actually less commonly in the hunger period than before or after. In the posthunger months there was a tendency for increase in stillbirths but this was probably related to a concurrent rise in the percentage of premature births. Data concerning congenital malformations showed a slight increase but unfortunately the small number of properly timed pregnancies rendered the difference of no statistical significance. Judged solely by the percentage of mothers nursing their babies during the hunger period, lactation was singularly unaffected.

These observations are surprising in view of the numerous studies suggesting a significant and clearcut relationship between maternal nutrition and almost all aspects of the course and product of pregnancy. In explanation, it is suggested as being most likely that the nutritional circumstances of the Dutch situation were not comparable to those of other studies in American, Canadian, and British obstetric clinics. It is stated that the Dutch women had been reasonably well fed until subjected to a brief period of acute general undernutrition. By contrast, the other studies usually involved women who had nourished themselves more or less indefinitely, as a rule, upon diets adequate in calories but insufficient in certain individual food elements.

ENVIRONMENT AND FOOD INTAKE

It has been common observation that the diets of inhabitants of tropical and semitropical climates differ considerably from those of temperate regions, and in turn from those of arctic climates. This would seem evident not only in the caloric intake of the inhabitants but in the proportion of carbohydrate, protein, and fat consumed. It seems probable that these variations are due in large part to eating and work habits, characteristic of the region, which are conditioned by climate and the food crops that can be produced locally. Even within the United States with the most highly developed means for transportation of perishable foods, the influence of availability of local food crops is evident in eating habits.

There have been no controlled studies, however, to determine whether climate as such greatly influences the choice of diet of individuals with a common background of eating habits and required to perform similar work. Requirements for a test experiment of this kind would necessitate mass displacements of men existing under regimented work and habit conditions, and an independent source of food supply. Such were provided by the recent global war.

R. E. Johnson and R. M. Kark (*Science* **105,** *378 (1947)*) have reviewed and reported food consumption data taken from records obtained in temperate, mountain, desert, jungle, subarctic, and arctic areas of North America, Europe, and Asia. The subjects were healthy North American soldiers transported to these regions, whose food intakes were studied only when full acclimatization to the new environment had occurred, and during periods when a wide variety of food was available to permit a free selection. From 50 to 200 men constituted a group for averaging data.

The investigators presented data showing a linear relation between voluntary caloric intake and the environmental temperature. The intake varied from 3100 calories in desert regions (92°F.) to 4900 calories in the Arctic (−30°F.). Only about 400 calories of this difference can be explained by the known change in basal metabolic rate in these different environments. Since the men had a similar weight and performed essentially the same work, this difference in caloric intake was believed dependent on the greater heat required to maintain thermal equilibrium in the Arctic, and on the lesser efficiency in performing the same task due to heavy clothing and equipment. No statement was made concerning possible weight gain of the soldiers in the Arctic, though an accompanying table indicates a somewhat greater body weight of the Arctic troops (73 kg. versus 65.5 to 70 kg.).

Of further interest were the data illustrating the proportion of calories supplied by protein, fat, and carbohydrate in these chosen diets. There was no definite shift in these proportions in the different regions, although possibly a little less fat was consumed in the tropics. Protein supplied 11 to 13 per cent of the calories, fat 33 to 43 per cent, and carbohydrate 44 to 54 per cent. There is little doubt that different diets would of necessity have developed if only the local food supply were available; otherwise it appears that early food habits determine the choice of diet rather than the environment.

TRYPTOPHANE REQUIREMENT OF THE HUMAN INFANT

Interest in the amino acid, tryptophane, is far from static. It is a dietary essential for man as well as for the rat (W. C. Rose, *Proc. Inst. Med. (Chicago)* **15,** *24 (1944)*) and there is evidence that it acts as a precursor of nicotinamide (*Nutrition Reviews* **4,** *334 (1946)*).

In man, 6 to 9 mg. of tryptophane per day

are required to produce a restoration of nitrogen equilibrium (*Nutrition Reviews* **3,** *208 (1945)*). It has been stated that the protein requirements of the infant are about five times greater than those of the adult human being. It is not known whether this excessive demand is due to the rapidity of growth of the organism or whether the increased requirements for nitrogen are created by an increase in all of the amino acids or by a limiting effect caused by higher demands for one or several of the amino acids. In an effort to answer this question, A. A. Albanese and co-workers (*Bull. Johns Hopkins Hosp.* **80,** *158 (1947)*) report a study of protein metabolism in infants, particularly as to their requirement for tryptophane.

Observations were carried out on 3 normal, healthy, white, male infants, 5 to 12 months of age, who were given the deficient diets and five feedings daily at the rate of about 100 calories per kilogram of body weight. The diet periods were consecutive and varied in duration from three to seven days. The diets were supplemented with 50 mg. of ascorbic acid and 15 drops of oleum percomorphum daily. Subjects were immobilized by use of abdominal restraints. The protein moiety of the tryptophane deficient diet was prepared by sulfuric acid hydrolysis of casein, as previously described by the authors (A. A. Albanese, L. E. Holt, C. N. Kajdi, and J. E. Frankston, *J. Biol. Chem.* **148,** *299 (1943)*). An attempt was made to establish minimal tryptophane requirements under these dietary conditions and, as a result, the addition of tryptophane to the diet was made stepwise and varied from 0.5 to 1.0 per cent of the protein content. Brewers' yeast was used instead of a mixture of the synthetic vitamins, and tryptophane derived from this source appears to be approximately 6 mg. per gram. Thus it is possible to calculate roughly the amount of tryptophane provided by the diets per kilogram of infant body weight.

The urine and stools were collected and analyses were carried out by the usual methods. From the data presented it is evident that in the absence of dietary tryptophane all 3 subjects underwent a marked decrease in the daily gain in weight and that a decrease in nitrogen retention occurred. Interestingly enough, the decrease in weight preceded the decrease in nitrogen retention in all instances. Body weight increased when tryptophane was again added to the diet. Thus, it appears that tryptophane is an essential dietary constituent for the infant. Anorexia also was noted during the period in which the deficient diets were administered but this disappeared promptly with addition of tryptophane.

It is suggested that the tryptophane requirement of an infant must lie between 23 and 40 mg. per kilogram of body weight or approximately five times the adult need. In all subjects on low-tryptophane intake, the output in the urine fell below normal but returned to normal levels promptly on restoration of this factor to the diet. This would suggest that the concentration of tryptophane in the urine might serve as a criterion for estimation of the dietary requirement of this amino acid.

Of considerable interest were the changes in the protein content of the blood serum. Within ten days on a tryptophane-poor diet, an appreciable hypoproteinemia developed which, for the most part, was reflected in a decrease in the concentration of albumin; the concentration of hemoglobin and of amino acid nitrogen in the blood was not influenced by the brief period of tryptophane deficiency. In this study, the measurement of the nitrogen retention, the rate of gain in body weight, the rate of output of tryptophane in the urine, and the concentration of plasma protein indicated that infants 5 to 12 months of age require about 30 mg. of l(−)-tryptophane per kilogram of body weight for normal growth. On a weight basis, the tryptophane, like the nitrogen, needs of infants are about five times as great as those of the adult.

In studies on the adult, experimental diets deficient in tryptophane had no effect on the plasma protein even after a period of

six weeks (L. E. Holt *et al.*, *Proc. Soc. Exp. Biol. Med.* **48,** *726 (1941)*). These results can be contrasted with a rapid loss of plasma protein induced in the infant by diets deficient in tryptophane. In the light of these experiments it appears dangerous to interpolate the dietary needs of infants from those of adults or animals.

PLASMA VITAMIN A AND CAROTENE LEVELS IN INFECTIOUS HEPATITIS

The relative ease with which serum or plasma determinations of vitamin A may be made has resulted in accumulation of a mass of data on the variations of these substances in disease in man. Estimations of the blood levels of vitamin A and carotene are of value in the clinical diagnosis of vitamin A deficiency, for in patients manifesting clinical signs of avitaminosis A the vitamin A levels are exceedingly low (*Nutrition Reviews* **3,** *38 (1945)*). Knowledge of the vitamin A blood values is also useful in several other clinical conditions, most of which are related to abnormalities of absorption or of liver function. For example, in active sprue low plasma carotenes may be of diagnostic value (*Ibid.* **3,** *306 (1945)*) and in cirrhosis of the liver there is a decreased concentration of both vitamin A and carotene in the blood (*Ibid.* **1,** *78 (1943)*).

A. D. Harris and T. Moore (*Brit. Med. J.* **1,** *553 (1947)*) report a study of the variations in vitamin A and carotene levels in 32 military patients, 30 of whom suffered from infectious hepatitis and 2 from arsenotherapy jaundice. The subjects ranged in age from 18 to 41 years and were fed a "normal diet" without restriction of fats and without addition of extra protein or vitamins during the period of observation. The vitamin A determinations were made by the Carr-Price reaction with a suitable correction for the color developed due to the carotenes. In the earliest periods of the hepatitis (zero to four days after hospital admission) the plasma vitamin A was depressed. The mean value for plasma vitamin A during this period was 54.6 I.U. per 100 ml. as contrasted to a mean value of 118 I.U. for a group of 41 healthy adults. The vitamin A concentration in the plasma showed a gradual rise following admission to the hospital until after one month it had reached a mean level of approximately 124 I.U. per 100 ml. Simultaneous observations on the carotene content of the plasma also indicated an increase in the concentration as the disease subsided. The initial low values for plasma vitamin A were independent of the preceding anorexia, but there was a statistically significant association between the lower vitamin A concentrations and fever or increasing age of the patient.

An interesting association existed between the time of increase of plasma vitamin A in infectious hepatitis and other tests of liver function. All of the vitamin A determinations were made during the icteric stage of the hepatitis, inasmuch as the diagnosis of infectious hepatitis was not usually made before the appearance of jaundice. The serum vitamin A levels increased from the time of admission, a rise which was found to parallel the simultaneous improvement in the hippuric acid synthesis test of liver function and, also, generally paralleled the return of the plasma prothrombin time to normal. At the same time, however, the serum bilirubin was found to increase after admission and the highest values were obtained on the fifth to ninth day. These results are consistent with the concept that the maximum liver damage in infectious hepatitis occurs during the pre-icteric stage.

Vitamin A tolerance tests, in which the plasma level was estimated following oral dosing with 96,000 I.U. of vitamin A, sometimes indicated a failure of the plasma level to rise after such administration. In 3 patients with hepatitis there was an increased

loss of vitamin A in the stools after the oral administration of massive doses. A repetition of the test as the patients recovered revealed that no vitamin A could be demonstrated in the feces during later convalescence. Similar doses administered to normal subjects did not give rise to detectable amounts of vitamin A in the stools. It appears, therefore, that in some cases of infectious hepatitis there is interference with the absorption of vitamin A during the early stages of the illness.

The authors point out that many months of a deficient intake of vitamin A are required to produce definite lowering of the concentration of this factor in the plasma. Anorexia or defective absorption could not therefore account for the decreased plasma levels observed in infectious hepatitis. In fatal cases of hepatitis the liver was found to contain adequate reserves of vitamin A which were sufficient to maintain normal plasma concentrations in healthy individuals. The authors conclude, therefore, that some failure in the mechanism of the release of vitamin A from the liver to the plasma must be strongly suspected in infectious hepatitis. It is suggested that there may be an interference with the hydrolysis of vitamin A esters and, therefore, failure to liberate free vitamin A alcohol from the liver into the blood stream.

In an addendum to this paper K. J. W. Craik, S. J. Macpherson, and A. D. Harris (*Brit. Med. J.* **1,** *558 (1947)*) report the results of dark adaptation determinations on a group of 37 service men with infectious hepatitis. Although dark adaptation proved to be reduced from the normal, this test did not prove as satisfactory a measure of vitamin A abnormality as direct blood determinations.

Studies such as these should enhance our knowledge of the physiology of absorption, storage, and release of vitamin A in man. Furthermore, they may lead to a more rational understanding of the altered metabolism in disease and thereby provide a sounder basis for therapy.

BODY FLUID CHANGES IN LIVER DISEASE

The movement of water between the various body compartments was once relatively easily explained by changes in osmotic and oncotic pressures. As a basis for understanding edema and diuresis this explanation has never been entirely satisfactory, and recent work has pointed to other factors especially in "hunger edema" (*Nutrition Reviews* **4,** *317 (1946)*). Relevant to this problem is the work of D. H. Labby and C. L. Hoagland (*J. Clin. Invest.* **26,** *343 (1947)*) who found storage of water in 14 young male patients with acute infectious hepatitis. A large series of measurements was made at intervals during each patient's course. These included body weight, hematocrit, thiocyanate space, plasma volume, blood volume, plasma proteins, serum bilirubin, bromsulfalein excretion, thymol turbidity, and plasma and urine chlorides. A water tolerance test was used in which the four hour urine excretion was measured after the ingestion of 1500 ml. of water.

In all patients the thiocyanate space (a measurement of extracellular fluid) and blood volume were greater during the acute stage of the disease than during convalescence and in most cases the change to a smaller thiocyanate space was gradual and progressive, being usually greatest in the first week or so of convalescence. Neither edema nor ascites was observed in any of these patients. The water tolerance test likewise showed a progressively larger excretory volume during convalescence. Plasma and urine chloride values were both below normal in the acute phase of the disease and both returned to or toward normal

as the thiocyanate space diminished during convalescence.

As the authors point out, their measurements confirm the clinical studies of C. M. Jones and F. B. Eaton (*New Engl. J. Med.* **213,** *907 (1935)*), who in addition stressed the favorable prognostic significance of diuresis in severe liver disease.

Any relation of the total plasma protein concentration to the fluid shifts is dismissed by the authors because no constant relationship between the two could be demonstrated. Inspection of the data, however, reveals a moderately but definitely lowered albumin concentration in many instances, usually rising at about the same time as the reduction in thiocyanate space. It would appear that this relationship of albumin concentration (the osmotically active plasma protein) to fluid movements might be more than fortuitous. Which is cause and which effect cannot be ascertained.

In explaining the retention of water during the acute phase of the disease the authors refer to their own work and that of others which suggests that a decreased inactivation of an antidiuretic hormone may occur in liver disease. The bioassay for this principle in the urine of these 14 patients will be awaited with interest.

PROTEIN HYDROLYSATES IN TREATMENT OF PEPTIC ULCER

The abnormal protein metabolism which occurs during convalescence after trauma (*Nutrition Reviews* **3,** *253 (1945)*), the relation of protein to production of antibodies (*Ibid.* **4,** *279 (1946)*), and the role of protein in human nutrition have been reviewed (*Ibid.* **4,** *6 (1946)*). In addition, the interesting association of protein deficiency and peptic ulceration in animals has been described (T.-W. Li and S. Freeman, *Gastroenterology* **6,** *140 (1946)*).

Peptic ulcer in man is a common disease and for it, as for most diseases of unknown etiology, methods of treatment are numerous. Most clinicians agree that treatment should be directed toward keeping gastric acidity as low as possible at all times. Methods usually employed to accomplish this consist of various forms of frequent feedings with milk, use of antacids of one type or another, and curbing of various psychic factors which influence gastric acidity. Although this plan of attack is not curative, in general it has been fairly successful when properly followed.

In 1945, Co Tui *et al.* (*Gastroenterology* **5,** *5 (1945)*) reported on a series of 30 patients with peptic ulcer who were given feedings of protein hydrolysate and dextrimaltose at two hour intervals. The mixture yielded from 0.5 to 0.8 g. of nitrogen and from 40 to 50 calories per kilogram of body weight per day. All of the patients experienced relief of distress within twenty-four hours and subsequent roentgenograms revealed evidence of rapid improvement in the ulcers.

Although the number of cases was small and the follow-up studies incomplete, this report received considerable publicity. The authors suggested that the beneficial effects of the protein hydrolysate might be due to the buffering action of the amino acids in addition to a high intake of nitrogen.

Several reports of attempts to study this suggested form of therapy further have been made; two of these deserve mention. S. T. Kimble (*Gastroenterology* **8,** *467 (1947)*) employed an enzymatic protein hydrolysate derived from beef, 1 liter of a 6 per cent solution of which contained 60 g. of protein. Approximately 50 per cent of the nitrogen was free amino nitrogen and 1 per cent was ammonium nitrogen. The pH was 6.5 and 100 ml. of the solution would neutralize 50 ml. of tenth-normal solution of hydrochloric acid. The study included 13 cases of duodenal ulcer, one of combined duodenal and

gastric ulcers, and one of gastric ulcer alone. In 11 of the cases a nasal rubber catheter was inserted into the cardiac portion of the stomach and through the tube, which remained in place for seven days, a continuous drip of protein hydrolysate, alternated with equal parts of whole milk and 20 per cent cream, was administered. During each twenty-four hours the 11 patients received 1500 ml. of hydrolysate (90 g. protein) and 1800 ml. of milk and cream. Four patients were given 1500 ml. of hydrolysate daily for seven to ten days by the intravenous route. In addition, 3 fluid ounces (90 ml.) of milk and cream were given hourly during the day. The 4 patients who received the protein hydrolysate solely by venoclysis at the end of the seven to ten days were put on a regular ulcer regimen. In 9 of the 15 cases the roentgenologic evidence of ulcer disappeared; 5 patients showed marked improvement and one did not respond to treatment.

H. H. Hodges (*Gastroenterology* **8**, *476 (1947)*) studied 18 patients with duodenal ulcer, 4 with gastric ulcer, 3 with gastrojejunal ulcer, and one with esophageal ulcer. Therapy consisted of administration of a mixture of equal parts of an enzymatic hydrolysate of casein and dextrimaltose. The dextrimaltose was considered to have no special value other than that of supplying carbohydrate and calories (3.9 calories for each gram). Between 650 and 800 g. of this mixture dissolved in 2000 to 2400 ml. of water were administered daily. Divided feedings were given as frequently as necessary to control symptoms, but at least every two hours while the patient was awake. The average sized patient ingested 750 g. of the mixture daily and was provided the nitrogen equivalent of 285 g. of protein, about 2900 calories, and about 7.5 g. of sodium chloride. Except in four instances no other food was given during the period of treatment (fourteen to twenty-one days) with the exception of vitamin supplements which were provided routinely. These 4 patients received 15 to 30 ml. of light cream or vegetable oil with each two hour feeding.

Complete relief of symptoms was obtained in 23 cases in an average of four and four-tenths days. Of the 23 cases in which the patients became asymptomatic, 12 had no roentgenologic evidence of ulcer activity after two or three weeks of treatment, 8 showed marked improvement, and 3 showed no roentgenologic evidence of improvement. Of the 20 cases in which roentgenologic evidence of healing or improvement occurred, in 5, ulcer symptoms recurred within one to five months after termination of therapy, and in one, surgical treatment of pyloric obstruction was necessary. Fourteen patients remained free of symptoms for from one to eight months. Hodges wisely pointed out that accurate evaluation of results from treatment with hydrolysate in this series is difficult and is largely a matter of clinical impression.

It is well known that many patients suffering from peptic ulcer are relieved of symptoms completely by just staying in bed and that most peptic ulcers can be controlled by rest in bed plus frequent feedings of milk, cream, and antacids. Of the recent studies on the use of protein hydrolysates in the treatment of peptic ulcer, adequate controls, having an equal number of patients treated with unhydrolyzed protein, have generally been lacking. Frequently, patients who have peptic ulcer have remissions which may last for years whether or not they receive treatment. Recurrences commonly are precipitated by undue nervous tension or fatigue and by many other factors in the environment. When dealing with such a capricious disease certainly the physician should be careful in interpreting the value of any therapy in a small series of cases in which the patients are observed for a short period. There are many experts in the field of radiology who do not feel that the activity of an ulcer can be determined solely by the roentgenologic appearance of the lesion.

Here is another difficulty in interpretation which the investigator encounters in attempting to evaluate results.

On the basis of data now available it does not appear that protein hydrolysates in treatment of peptic ulcer are of any greater value than the other commonly used methods. It would appear that many more patients must be observed for long periods of time, with proper controls, before this type of therapy can be considered useful in the clinical management of peptic ulcer.

GROWTH REQUIREMENTS OF PATHOGENIC PROTOZOA

Although a number of the free-living protozoa have been cultivated in culture mediums of chemically known composition, the nutritive requirements of pathogenic representatives of these microscopic, unicellular animals are almost wholly unknown. Since many widespread human diseases are caused by organisms of this group, a knowledge of their nutrition would be of considerable interest and might provide an insight into means for combating their deleterious effects.

H. Sprince and A. B. Kupferberg (*J. Bact.* **53,** *435* (*1947*)) studied this problem, using as the test organism the flagellate, Trichomonas vaginalis, a human parasite. G. Johnson and R. E. Trussell (*Proc. Soc. Exp. Biol. Med.* **54,** *245* (*1943*)) had previously shown that this organism could be grown and maintained in pure culture in a basal medium composed chiefly of peptone, liver infusion, maltose, cysteine, and unheated blood serum. Sprince and Kupferberg succeeded in replacing each of the major components of this crude medium, except blood serum, with purified materials. Their purified medium contains an enzymatic protein digest (Trypticase), sodium acetate, maltose, all of the known B-vitamins, the purine and pyrimidine bases, ribose, asparagine, ascorbic acid, sodium bicarbonate, and agar. This basal medium supports growth of the flagellate only if blood serum is added; until the essential factors furnished by the blood serum are identified, the essential components of the basal medium cannot be determined with certainty. When supplemented with blood serum, the medium supported sustained growth of the flagellate through sixty serial transfers, and permitted populations as high as 1600 organisms per cubic millimeter to be obtained.

Attempts were made by the same authors (H. Sprince and A. B. Kupferberg, *J. Bact.* **53,** *441* (*1947*)) to identify the components of blood serum necessary for growth. Repeated ether extraction of human blood serum at pH 7.1 to 7.3 separated the serum into two fractions, an ether soluble fraction, and the remaining ether insoluble residue, both of which were necessary for growth of the organism.

The active material in the ether soluble phase was heat stable and dialyzable, and could be replaced rather effectively by a mixture of lecithin, cholesterol, oleic acid, linoleic acid, ergosterol, alpha-estradiol, alpha-tocopherol, beta-carotene, and vitamin A. The chief effective component of this mixture appears to be linoleic acid, although other stimulatory substances are present in the ether extract.

The essential substance present in the ether insoluble fraction of blood serum could be partially, though not completely, replaced by addition of human serum albumin. Sustained, but not maximum growth was obtained with this replacement. It was obvious, however, that the ether insoluble phase also contained stimulatory factors in addition to its main active ingredient, serum albumin. The nature of these stimulatory factors is at present unknown, but it is evident that, with identification of the chief essential components of blood serum, the basis has been laid for the

complete elucidation of the nutritive requirements of this organism. The apparent requirement of the organism for an intact protein is interesting and unusual. It will be interesting to see if the serum albumin represents an actual nutritive requirement, or whether it is necessary only to detoxify the fatty acids required for growth, as reported by R. J. Dubos (*Proc. Soc. Exp. Biol. Med.* **63,** *56 (1946)*) and by B. D. Davis and R. J. Dubos (*Arch. Biochem.* **11,** *201 (1946)*) for several bacteria.

EFFECT OF DICUMAROL ON VITAMIN A DEFICIENT DUCKS

K. G. Wakim, K. K. Chen, and W. D. Gatch (*Surg. Gynec. Obst.* **76,** *323 (1943)*) showed that Dicumarol in large doses may have a toxic action on certain animals prior to the onset of hypoprothrombinemia. Hypoprothrombinemia resulting from the injection of this drug requires a latent period of approximately twenty-four hours. Dogs given 40 to 60 mg. of Dicumarol per kilogram of body weight intravenously usually died within an hour without showing any changes in either the prothrombin or the coagulation times. Such dogs may become dyspneic, have convulsions, and may develop coma preceding death.

During studies of malaria in ducks R. H. Rigdon and N. B. Varnadoe (*J. Lab. Clin. Med.* **32,** *532 (1947)*) observed that normal ducklings frequently died within three or four hours following oral administration of large doses of Dicumarol. The time of death following the oral administration of Dicumarol, furthermore, seemed to vary in ducks fed a vitamin A deficient ration when compared with birds fed a standard commercial ration.

A total of 149 ducks were used in these experiments. Various experimental groups were used in which ducks received vitamin A deficient diets, normal standard rations, and diets supplemented with added amounts of vitamin A. The quantity of Dicumarol given each day ranged from 50 to 200 mg. Birds fed a ration supplemented with vitamin A were more resistant to the acute lethal effects of Dicumarol than birds fed a ration low in this vitamin. Thus, in four experiments 17 of 31 ducks fed a vitamin A deficient ration were dead by the third day whereas only 4 out of 30 ducks fed a standard ration were dead at that time. Again, 5 of 17 ducks fed standard rations supplemented with vitamin A died as compared with 14 out of 20 which were fed only the standard ration. In another experiment 7 out of 15 ducks fed the vitamin A deficient ration (46.6 per cent) died whereas only 2 of 15 (13.3 per cent) fed the same ration supplemented with vitamin A succumbed, and 5 of 13 ducks (38.4 per cent) fed the standard ration were dead by the third day.

The observation that ducks fed large amounts of vitamin A are relatively resistant to large doses of Dicumarol is interesting because vitamin A is stored in the liver and Dicumarol has been found to produce necrosis in the liver of laboratory animals (Wakim *et al., loc. cit.;* R. K. Richards and R. Cortell, *Proc. Soc. Exp. Biol. Med.* **50,** *237 (1942)*). This action occurs before hypoprothrombinemia develops. Rigdon and Varnadoe could find no specific pathologic changes in the livers of the ducks given large doses of Dicumarol for as long as five days and offer no explanation as to the cause of acute death in their birds. They did observe that a 50 per cent decrease in the number of red blood cells occurred within two to four hours following the oral administration of large doses of Dicumarol; yet the plasma at this time did not show hemolysis.

It is obvious that the amounts of Dicumarol used in these experiments are greatly in excess of those used therapeutically in man. Consequently, there is

no reason to believe that these studies apply to the therapeutic use of this drug. Nevertheless, since the reserves of vitamin A in the liver are low in certain hepatic diseases it may be advisable to be cautious in the use of Dicumarol in such patients.

THE ENHANCEMENT OF VITAMIN A ABSORPTION

The therapeutic usefulness of vitamin A has been limited by its slow absorption, especially in certain disease states such as celiac disease, pancreatic fibrosis, and bile duct obstruction. By using an aqueous dispersion of fish liver oil, B. Kramer, A. E. Sobel, and S. P. Gottfried (*Am. J. Dis. Child.* **73,** *543 (1947)*) have so increased the rate of absorption of the vitamin that very high blood levels are obtained three hours after the oral test dose. This effect became even more important since both the above authors and G. Cienfuegos (*J. Pediatrics* **28,** *191 (1946)*) have shown that blood vitamin A levels rise little, if at all, after intramuscular injection of vitamin A esters. Kramer *et al.* did find a delayed peak of blood vitamin A, but only nine hours after the intramuscular injection of an aqueous dispersion of vitamin A acetate.

The aqueous dispersion of fish liver oil used for oral tolerance tests contained in addition to 6000 U.S.P. units of vitamin A and 1200 U.S.P. units of vitamin D in each 0.6 ml.: thiamine 1.8 mg., riboflavin 0.6 mg., pyridoxine 0.3 mg., nicotinamide 3 mg., calcium pantothenate 1.2 mg., ascorbic acid 60 mg., as well as an extract of rice polishings, choline, inositol, and biotin in unspecified amounts.

Vitamin A tolerance tests were conducted on 10 children (ages 1½ months to 12 years) administering orally 0.6 ml. of the dispersion in orange juice. These were compared in the same children with a previous and sometimes subsequent tolerance test using an equivalent amount of undispersed fish liver oil. Five of the children had the celiac syndrome with steatorrhea and with flat vitamin A curves following administration of fish liver oil. In contrast, following administration of the aqueous dispersion, the highest blood levels were observed at three hours, and in some instances persisted through six and even nine hours. The average maximum elevation was 142 micrograms per 100 ml. of plasma as compared with 34 micrograms when fish liver oil was used.

Similar evidence of more rapid absorption of the aqueous dispersion was seen in the 5 normal children who, although not having a flat tolerance curve to fish liver oil, had much higher three, six, and nine hour values following the aqueous dispersion, the average maximum elevation with fish liver oil being 133 micrograms per 100 ml. of plasma and with the aqueous dispersion 308 micrograms.

As a control, a dispersion using the same fish liver oil was given but without the vitamin B-complex or ascorbic acid. Such a preparation, however, required more of the stabilizer. In this instance much higher blood levels were attained but whether they were due to the increased amount of the stabilizer or to the absence of the other vitamins was not shown. The highest value was 1322 micrograms of vitamin A per 100 ml. of plasma. As a further control, an aqueous solution of the dispersion agent alone was administered. No rise in plasma vitamin A occurred.

The clinical data concerning the patients with the celiac syndrome are presented, but no mention is made of specific signs suggestive of vitamin A deficiency nor of any clinical effect of the administration of the aqueous dispersion of vitamin A.

NOTES

Vitamin A in the Diet of Bobwhites

R. B. Nestler (*Tr. Eleventh North American Wildlife Conf.* **176** (*1946*)) demonstrated the importance of vitamin A for the survival of bobwhite quail when raised on game farms. Studies conducted on over 2000 birds indicated that at least 3000 I.U. of the vitamin were required per pound of feed for optimum growth, whereas 6000 units per pound were needed for maintenance of egg production and hatchability, and 8000 units for survival of the breeders. Deficient birds showed characteristic avitaminotic symptoms, including severe ophthalmia, rhinitis, and visceral gout.

Transfer of vitamin A by the hen to the chicks diminished throughout the season, as evidenced by a drop from 146 units per gram in the liver of chicks in the first hatch to 66 units per gram in the last hatch. Livers of the latter group (from parents receiving 8000 units per pound of feed) contained less vitamin A than those of the first hatch from parents receiving no vitamin A. This may account for the poor success of second and third clutches of eggs from wild quail.

Survival of bobwhites during winter was impaired markedly by a low vitamin A diet, and was found to be as low as 30 per cent when the vitamin A content of the diet was 500 units per pound. Complete removal of the vitamin from the latter diet resulted in death of the birds in thirteen days, whereas those formerly on optimum levels of the vitamin survived for fifty days. These observations led the author to suggest that vitamin A deprivation may easily occur among wild birds to the point of extinction of large numbers of the game bird population. Since pen-fed quail do not select carotene-rich natural foods, and have to be trained to eat them, Nestler recommends that these foods be introduced to the quail before they are released from the game farms. Stocking of many areas with game birds depends in large measure upon the success of quail raising (387,000 birds were raised in 1940), and it appears that further research on the nutrition of this species is desirable so that proper management practices may be carried out.

A Pharmacologic Action of Thiamine

P. Shub, a Russian investigator, reports in a brief note (*Am. Rev. Soviet Med.* **4,** *476* (*1947*)) that thiamine was effective in the treatment of labor pains. Intramuscular injections were given in 60 mg. doses and if pain reappeared an additional 40 mg. were given. The analgesic effect appeared within ten to fifteen minutes and lasted till the end of labor in 4 per cent of the cases. In 51 per cent the effect was somewhat less, in 3 per cent it was of short duration, and in 5 per cent the injection was without effect.

The work reported was done at the Leningrad Central Institute of Gynecology and Obstetrics. No actual experimental protocols are reported in the note.

Distribution of Ascorbic Acid in the Blood

General agreement concerning the distribution of ascorbic acid between cells and plasma has not been reached, despite many studies. J. H. Roe, C. A. Kuether, and R. G. Zimler (*J. Clin. Invest.* **26,** *355* (*1947*)) have made precise measurements of the whole blood and plasma ascorbic acid concentrations of 50 medical students and 50 hospital patients with a variety of illnesses. In addition, similar studies were made on 52 guinea pigs fed diets containing known amounts of ascorbic acid.

In the group of normal individuals the plasma ascorbic acid concentrations were below those for whole blood when the whole blood concentration was less than 0.6 mg. per 100 ml., both were approximately equal for whole blood values between 0.6 and 0.9 mg. per 100 ml., while when the whole blood values exceeded 0.9 mg. per 100 ml., the plasma values were generally higher. In contrast, all the patients studied had more ascorbic acid in whole blood than plasma without relation to the actual whole

blood concentration (range 0.05 to 1.3 mg. per 100 ml.). The distribution of ascorbic acid in the blood of the guinea pig was similar to that in normal individuals.

Reviewing the literature, the authors found agreement with their results only in those instances in which the whole blood concentration was known. The reduction in plasma concentration in the patients studied was thought to represent a greater need for the vitamin, the plasma ascorbic acid being more available than that of the red cells. Such may be the case, but a number of alternative possibilities suggest themselves. Thus, lower plasma than whole blood values might result from impaired absorption, excessive excretion, or alteration in the cell-plasma equilibrium, as well as from the increased need the authors suggest. These possibilities should be investigated before a greater need for ascorbic acid is deduced from the data presented.

Rapid Estimations of Serum Albumin and Globulin

The concentration of serum albumin is often thought to be of more value in assessing nutrition status than is the estimate of total serum protein alone. The widely used procedure for determining albumin consists of a salt precipitation and subsequent nitrogen determination by the Kjeldahl technic. The method is time-consuming for use in nutrition surveys. A principle for the determination of serum albumin and globulin has been suggested by A. B. Lerner and C. P. Barnum (*Arch. Biochem.* **11,** *505 (1946)*) and appears to be adaptable to survey purposes.

This method is based upon the determination of the ultraviolet absorption of a diluted solution of the serum proteins in a spectrophotometer. The total protein concentration of the serum is estimated simultaneously by one of the rapid specific gravity procedures, such as that of O. H. Lowry and T. H. Hunter (*J. Biol. Chem.* **159,** *465 (1945)*). The serum albumin concentration may be calculated from the combined data.

To arrive at a convenient method of calculation, the authors made a number of approximations in deriving the formula which they propose. The effect of these simplifications was then tested by comparing the calculated albumin concentration with the albumin concentration of the same sample as estimated by the Kjeldahl procedure in a series of ten serums from pathologic cases. The differences between the results by the two methods was less than 10 per cent in eight of the ten samples which were tested. The greater discrepancies occurred in serum from patients with hepatic disease. Sulfa drugs were found to interfere with the spectrophotometric method. No data are presented on the comparison of values for serum albumin by the exact procedure outlined by the investigators with the results of some widely accepted technic. The principle outlined by Lerner and Barnum is worthy of a critical test under actual working conditions. The acceptance of unproved methods, however reasonable appears their basis, has been all too common in nutrition surveys in the past.

Recent Nutrition Books

Estimation of the Vitamins. Biological Symposia. Vol. 12. Edited by W. J. Dann and G. Howard Satterfield. The Jaques Cattell Press, Lancaster, Pa. 1947. Pp. 531.

Methods of Vitamin Assay. The Association of Vitamin Chemists, Inc. Interscience Publishers, Inc., New York. 1947 Pp. 189.

Practical Physiological Chemistry. Philip B. Hawk, Bernard L. Oser, and William H. Summerson. Twelfth Edition. The Blakiston Co., Philadelphia, Pa. 1947. Pp. 1323.

The Yearbook of Agriculture 1943–1947. Science in Farming. For sale by the Superintendent of Documents, Washington 25, D. C. Price $2.00. Pp. 934.

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